[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Infrared Spectra of N-Phosphorylated Derivatives of Amino Acids[1]

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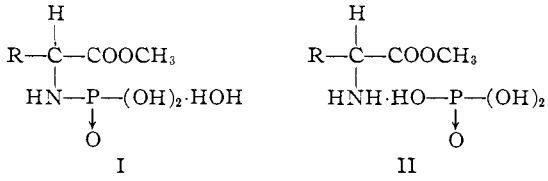
Infrared spectra of some phosphoramides prepared from α-amino acid derivatives are reported. Observed absorption bands in the regions of 10.62–10.80 μ and 11.38–11.54 μ are tentatively assigned as the contribution of N–P bonds in the $-NH-PO_3H_2$ group and the $-NH-\overset{\uparrow}{\underset{O}{P}}(OCH_2-)_2$ group, respectively. Additional absorption in the region of 13.30 μ by the latter group may also occur, but such a band is masked by the absorption of any phenyl group in the molecule.

Previous publications[3,4] reported the syntheses of a group of compounds of a type that may possess significant biological importance—N-phosphorylated amino acids and their derivatives. Infrared absorption data on a number of these compounds have been determined for us through the courtesy of Samuel P. Sadtler and Sons, Inc., Philadelphia, Penn. The series of spectra is not complete enough for an exhaustive analysis, but we are reporting this information now since no further syntheses are planned for the present time and as almost nothing has been published previously on the absorption characteristics of phosphoramides.

Possible Absorption Bands of N–P Bond in $-NH-PO_3H_2$ Group.—Considerable difficulty was encountered in obtaining crystalline products in the syntheses of N-phosphorylated amino acids, and the best characterized and purest preparations were those made from aromatic amino acids. The N-phosphorylated methyl esters of phenylalanine and tryptophan crystallized with one molecule of water of hydration I. These phosphoramide hydrates are isomeric with the corresponding phosphate salts II, but the isomers differ markedly in



physical, chemical and biological properties.[3] Comparison of the infrared spectra of the two isomeric pairs (Fig. 1A, 1B, 1D and 1E) shows that all the well defined bands of medium (M) or strong (S) absorption can be included in one of five groups.

A. Bands common to all four compounds: 3.53–3.57 μ (s), 3.80–3.86 μ (M), 4.32–4.35 μ (M), 5.75–5.76 μ (M), 6.55–6.70 μ (M), 6.90 μ (S), 7.26–7.28 μ (M), 7.68–7.72 μ (M), 8.0–8.15 μ (S), 8.65–8.70 μ (M–S), 9.50–9.63 μ (S), 11.65–11.95 μ (M).

B. Bands common only to the phenylalanine derivatives: 13.25–13.42 μ (M), 14.35–14.37 μ (M).

C. Bands common only to the tryptophan derivatives: 3.04–3.06 μ (M), 3.36–3.38 μ (S), 13.53–13.64 μ (S).

D. Bands common to N-phosphoryl amino acid ester hydrates: 6.22 μ (M), 9.25–9.26 μ (S), 10.62–10.80 μ (S), 12.60–12.80 (M).

E. Bands common to the phosphate salts of amino acid esters: 8.97–9.08 μ (S), 11.20–11.22 μ (M).

When the band for the water of hydration (6.22 μ) is eliminated from consideration, only three moderately strong or strong bands (group D) remain for possible assignment to the phosphoramide linkage. When the spectrum of N-phosphoryl-DL-phenylalanine (Fig. 1C) is included in the comparison the two bands 10.62–10.80 μ and 12.6–12.8 μ are the only regions of comparable absorption common to all $-\underset{|}{CH}-NH-PO_3H_2$ compounds. The spectrum of a phosphoramide of an aromatic amine, N-(*p*-chlorophenyl)-amido phosphoric acid (Fig. 1R), has only the 10.62–10.80 μ band in common with phosphoramides of aliphatic amino groups.

Possible Absorption Bands of the N–P Bond in $-NH-\overset{\uparrow}{\underset{O}{P}}(OCH_2-)_2$ Group.—N-Dibenzylphosphoryl derivatives of amino acid esters, amino acid amides and aromatic amines were prepared as intermediates to be hydrogenated with the hope of obtaining the corresponding N-phosphoryl compounds. Spectra of representative N-dibenzylphosphoryl derivatives are reproduced (Fig. 1F–1K, 1Q). Other spectra not presented here but available from Sadtler Research Laboratories, Philadelphia, Penn., include the N-dibenzylphosphoryl derivatives of DL-alanine methyl ester, DL-leucine methyl ester, DL-threonine methyl ester, L-glutamic acid dibenzyl ester, L-tyrosine ethyl ester, L-tyrosine ethyl ester (in which the phenolic group as well as the α-amino group is phosphorylated), L-tyrosine benzyl ester, L-lysine methyl ester (N^{α}, N^{ϵ}-substituted), L-arginine methyl ester (N,N′-substituted), and *p*-toluidine.

When the bands of N-dibenzylphosphoryl derivatives of amino acid esters for which there are no obvious assignment (Table I) are compared with those of the N-diethylphosphoryl derivatives of glycine ethyl ester (Fig. 1–O) and DL-alanine methyl ester (Fig. 1P) and of the N-dibenzylphosphoryl derivatives of glycine amide (Fig. 1L), DL-alanine amide (Fig. 1M), *p*-chloroaniline (Fig. 1R) and *p*-toluidine, only the one in the region of 11.38–11.59 μ is found to be common to all the $-NH-\overset{\uparrow}{\underset{O}{P}}(OCH_2-)_2$ containing compounds.

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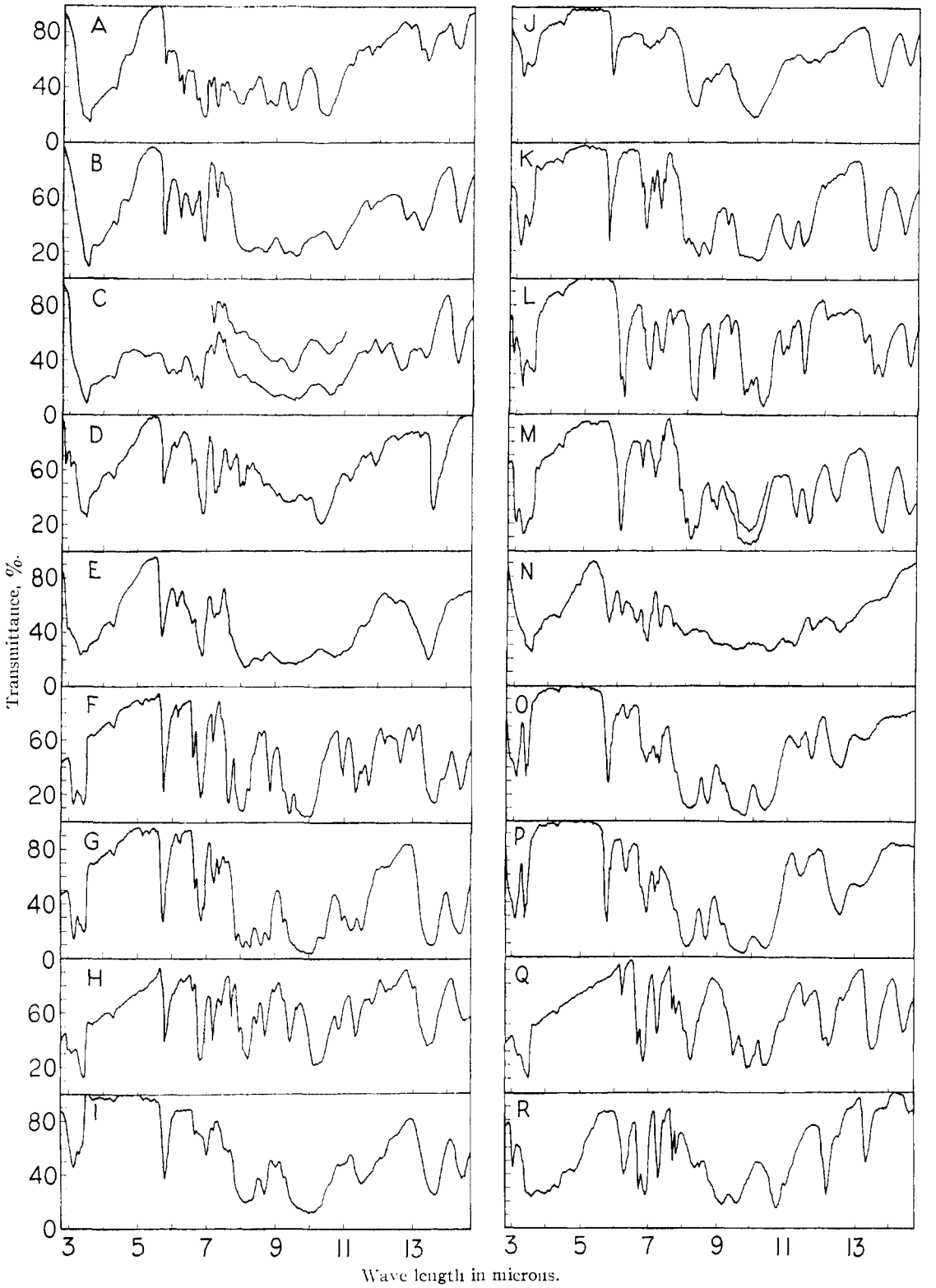

Fig. 1.—Infrared absorption spectra of phosphoryl compounds: A, DL-phenylalanine methyl ester phosphate; B, N-phosphoryl-DL-phenylalanine methyl ester hydrate; C, N-phosphoryl-DL-phenylalanine hydrate; D, DL-tryptophan methyl ester phosphate; E, N-phosphoryl-DL-tryptophan methyl ester hydrate; F, N-dibenzylphosphoryl-DL-phenylalanine methyl ester; G, N-dibenzylphosphoryl-DL-phenylalanine benzyl ester; H, N-dibenzylphosphoryl-DL-tryptophan methyl ester; I, N-dibenzylphosphoryl-DL-valine methyl ester; J, N,N′-bis-(dibenzylphosphoryl)-L-cystine dimethyl ester; K, N-dibenzylphosphoryl glycine ethyl ester; L, N-dibenzylphosphoryl glycine amide; M, N-dibenzylphosphoryl-DL-alanine amide; N, glycine ethyl ester phosphate; O, N-diethylphosphoryl glycine ethyl ester; P, N-diethylphosphoryl-DL-alanine methyl ester; Q, N-dibenzylphosphoryl-*p*-chloroaniline; R, N-(*p*-chlorophenyl)-aminophosphoric acid.

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The spectrum of $H_2N-P-(OCH_2CH_3)_2$ obtained by Holmstedt and Larsson[5] shows an unassignable band in the region of 13.2–13.3 μ. A definite band, though not too strong, 13.2–13.4 μ, occurs in the two compounds in our series which have no aromatic nuclei (Fig. 1–O and 1P). In the case of the other compounds, any absorption at this wave length by the N–P bonds present would be masked by the strong, broad aromatic bands around 13.5 μ.[6] This suggested assignment is consistent with the spectrum of the phosphate salt of glycine ethyl ester (Fig. 1N) which shows no absorption in this region.

A comparison of the absorption of N-dibenzylphosphoryl amino acid esters and the corresponding products formed upon hydrogenation (Table I) indicates the formation of N-phosphorylated compounds was successful. Hydrogenolysis of N-dibenzylphosphoryl amino acid benzyl esters caused one more change not observed with the methyl or ethyl esters—a shift of a band in the region of 5.78 μ (sharp and strong) to 5.88 μ (medium and broad). This shift and change in the shape of the band is a characteristic difference to be expected upon conversion of a carboxylic ester to the acid.[6]

TABLE I

COMPARISON OF CHARACTERISTIC ABSORPTION BAND OF N-DIBENZYLPHOSPHORYL AND N-PHOSPHORYL AMINO ACID ESTERS

N-Dibenzylphosphoryl cmpd. Region of band, μ	Assignment	N-Phosphoryl cmpd. Region of band, μ	Assignment
3.12–3.19	C–H[4]	None	
None		6.22	Water of hydration[6]
9.20–9.26	?	9.20–9.26	?
			O ↑
None		9.50–9.63	—P(OH)$_2$ group (presumably)
9.50–10.4 (broad)	P–O–C (aliphatic)[5,7,8]	None	
None		10.62–10.80	N–P bond (presumably)
10.90–11.0	?	None	
11.38–11.54	N–P bond (presumably)	None	

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Experimental

Equipment and Measurements.—The absorption spectra between 2 and 15 μ were obtained on a Baird Associates double beam Infrared Recording Spectrophotometer, using a sodium chloride prism. The solid samples were dispersed in "Nujol" (mineral oil), placed between sodium chloride plates, and scanned using plain "Nujol" in the reference beam to compensate for the Nujol used with the sample. The liquid samples were placed directly in a demountable cell consisting of two sodium chloride discs separated by a 0.01 mm. gasket and scanned.

Preparation of Amino Acid Ester Phosphates.—The hydrochlorides of amino acid esters were neutralized in dry methanol with sodium methoxide and enough ether added to complete the precipitation of the sodium chloride. To the filtrate was added the appropriate amount of phosphoric acid (86%). The phosphate salt which separated out was recrystallized from methanol and ether; glycine ethyl ester phosphate, m.p. 230–231° (uncor.).

Anal. Calcd. for $C_4H_{12}O_6NP$ (201): N, 6.96; P, 15.4. Found: N, 6.47; P, 15.2.

DL-Tryptophan methyl ester phosphate, m.p. 204–205° (uncor.).

Anal. Calcd. for $C_{12}H_{17}O_6NP$ (302): N, 9.27; P, 10.2. Found: N, 9.16; P, 9.7.

Preparation of N-Diethylphosphoryl Amino Acid Esters. The reaction between amino acid esters and diethylphosphoryl chloride was carried out in an analogous manner to that described for their reaction with dibenzylphosphoryl chloride[3] (Procedure a). After standing overnight, the reaction mixture was treated with ether until no more triethylamine hydrochloride precipitated. The solvent was removed from the filtrate and the oily residue distilled *in vacuo*. N-Diethylphosphoryl glycine ethyl ester: yield 51%; b.p. 126–132° (0.5 mm.); $n^{25}D$ 1.4340.[9] N-Diethylphosphoryl DL-alanine methyl ester: yield 69%; b.p.118–119° (0.5 mm.); $n^{25}D$ 1.4332.

Anal. Calcd. for $C_8H_{18}O_5NP$ (239): N, 5.9; P, 13.0. Found: N, 6.57; P, 13.7.

Other compounds used for the spectra analyses were prepared according to methods described previously.[3,4,10,11]

AUSTIN, TEXAS

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